PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

LJH

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To:
TOWNSEND AND TOWNSEND AND CREW LLP
Attn. Lockyer, Jean M.
Two Embarcadero Center, 8th Floor
San Francisco, CA 94111
UNITED STATES OF AMERICA

015280-5000 00PC

Applicant's or agent's file reference 15280-5000PC	Date of mailing *(day/month/year)* 23/08/2005 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2004/039617	International filing date *(day/month/year)* 24/11/2004
Applicant THE GOVERNMENT OF THE UNITED STATES ...	

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 art 19 amendment	Authorized officer Stefanie Büchler

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

DOCKETED BY 00
previously filed EDS in US
DOCKETED BY 00
previously

search rept recd
DOCKETED BY 00
previously

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
15280-5000PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2004/039617	24/11/2004	25/11/2003
Applicant		
THE GOVERNMENT OF THE UNITED STATES ...		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      [ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [X] **Certain claims were found unsearchable** (See Box II).

3. [X] **Unity of invention is lacking** (see Box III).

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. 8A
      [X] as suggested by the applicant.
      [ ] as selected by this Authority, because the applicant failed to suggest a figure.
      [ ] as selected by this Authority, because this figure better characterizes the invention.
   b. [ ] none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/039617

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [X] in computer readable form

   c. time of filing/furnishing

   [ ] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [X] furnished subsequently to this Authority for the purpose of search

2. [X] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/039617

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 53-63 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-74

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-74

An antibody that specificaly binds CD22, said anti-CD22 antibody having a variable light (VL) chain comprising three complementary determining regions designated CDRs 1, 2, and 3, wherein said CDR1 has a sequence selected from the group consisting of SEQ ID NO: 7, 8, 9, and 10; and subject-matter related thereto.

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2. claims: 75-105

A Pseudomonas exotoxin A or a cytotoxic fragment or mutant thereof, wherein said PE has a glycine, alanine, valine, leucine, or isoleucine in place of arginine at the position corresponding to position 490 of SEQ ID NO: 24; and subject-matter related thereto.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/039617

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/28 C07K19/00 C12N15/13 C12N15/62 A61K39/395
C07K14/21 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 03/027135 A (THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY O) 3 April 2003 (2003-04-03) the whole document	1-74
Y	SALVATORE G ET AL: "Improved cytotoxic activity cell lines and fresh leukemia cells of a mutant anti-CD22 immunotoxin obtained by antibody phage display" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 8, April 2002 (2002-04), pages 995-1002, XP002971541 ISSN: 1078-0432 the whole document	1-74

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 April 2005	23. 08. 2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Morawetz, R

2

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHOWDHURY P S ET AL: "IMPROVING ANTIBODY AFFINITY BY MIMICKING SOMATIC HYPERMUTATION IN VITRO" NATURE BIOTECHNOLOGY, NATURE PUB. CO, NEW YORK, NY, US, vol. 17, June 1999 (1999-06), pages 568-572, XP000918985 ISSN: 1087-0156 the whole document	1-74
Y	WO 00/73346 A (THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE) 7 December 2000 (2000-12-07) the whole document	1-74
Y	BEERS R ET AL: "Immunotoxins with increased activity against epidermal growth factor receptor vIII-expressing cells produced by antibody phage display" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 6, no. 7, July 2000 (2000-07), pages 2835-2843, XP002176480 ISSN: 1078-0432 the whole document	1-74
T	HO MITCHELL ET AL: "In vitro antibody evolution targeting germline hot spots to increase activity of an anti-CD22 immunotoxin." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 7 JAN 2005, vol. 280, no. 1, 7 January 2005 (2005-01-07), pages 607-617, XP002326657 ISSN: 0021-9258 the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/039617

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03027135 A	03-04-2003	CA 2461351 A1	03-04-2003
		EP 1448584 A2	25-08-2004
		WO 03027135 A2	03-04-2003
		US 2005118182 A1	02-06-2005
WO 0073346 A	07-12-2000	AU 5303700 A	18-12-2000
		CA 2374398 A1	07-12-2000
		EP 1180123 A1	20-02-2002
		JP 2003502030 T	21-01-2003
		MX PA01011950 A	06-05-2002
		WO 0073346 A1	07-12-2000

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

LJH PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

015280-500000 PC

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below 11/23/05

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/039617	24.11.2004	25.11.2003

International Patent Classification (IPC) or both national classification and IPC
C07K16/28, C07K19/00, C12N15/13, C12N15/62, A61K39/395, C07K14/21, A61K47/48

Applicant
THE GOVERNMENT OF THE UNITED STATES ...

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

11/03/05

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

9/25/05

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Morawetz, R

Telephone No. +49 89 2399-8155

Form (PCT/ISA/237) (Cover Sheet) (January 2004)

response to written opinion
DOCKETED BY

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/039617

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-105

because:

☒ the said international application, or the said claims Nos. 53-63 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☒ the claims, or said claims Nos. 1-74 are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 75-105

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-74

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-74
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-74
Industrial applicability (IA)	Yes:	Claims	1-52, 64-74
	No:	Claims	53-63: no opinion

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 53-63 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

2. No opinion will be established for claims 75-105, since no international search report has been established for said claims.

3. Present claims 1-74 relate to an extremely large number of possible antibodies. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small portion of the antibodies claimed. In the present case, the claims thus lack support and the application lacks disclosure. Consequently examination has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the antibodies disclosed in the examples of underlying application.

Re Item IV

Lack of unity of invention

Rule 13 PCT stipulates that the international application shall relate to one invention only or to a group so linked as to form a single general inventive concept. Where a group of inventions is claimed in one and the same international application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding "special technical features", i.e. technical features that define a novel and inventive contribution over the prior art (Rule 13.2 PCT).

The first group of inventions is directed to an antibody that specifically binds CD22, said anti-CD22 antibody having a variable light (V_L) chain comprising three complementary determining regions designated CDRs 1, 2, and 3, wherein said

CDR1 has a sequence selected from the group consisting of SEQ ID NO: 7, 8, 9, and 10. The second group of inventions is directed to a Pseudomonas exotoxin A or a cytotoxic fragment or mutant thereof, wherein said PE has a glycine, alanine, valine, leucine, or isoleucine in place of arginine at the position corresponding to position 490 of SEQ ID NO: 24. The special technical feature of the first invention is the anti-CD22 antibody showing increased affinity to CD22. The special technical feature of the second group is the Pseudomonas exotoxin A showing an increased activity. There is no technical relationship between these inventions let alone one which would involve these special technical features. These claims thus lack unity a priori.

Due the abscence of a single general inventive concept between the two inventions and due to the fact that no "special" technical feature (Rule 13.2 PCT) could be identified to provide a linking concept between the different groups of inventions, each invention has to be seen as individual contribution to the art, which is not linked with the other invention by a single general inventive concept. Consequently there is lack of unity.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Cited documents

Reference is made to the documents cited in the international search report.

D1: WO 03/027135 A (THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY O) 3 April 2003 (2003-04-03)

D2: SALVATORE G ET AL: "Improved cytotoxic activity cell lines and fresh leukemia cells of a mutant anti-CD22 immunotoxin obtained by antibody phage display" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 8, April 2002 (2002-04), pages 995-1002, XP002971541 ISSN: 1078-0432

D3: CHOWDHURY P S ET AL: "IMPROVING ANTIBODY AFFINITY BY MIMICKING SOMATIC HYPERMUTATION IN VITRO" NATURE

BIOTECHNOLOGY, NATURE PUB. CO, NEW YORK, NY, US, vol. 17, June 1999 (1999-06), pages 568-572, XP000918985 ISSN: 1087-0156

D4: WO 00/73346 A (THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE) 7 December 2000 (2000-12-07)

D5: BEERS R ET AL: "Immunotoxins with increased activity against epidermal growth factor receptor vIII-expressing cells produced by antibody phage display" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 6, no. 7, July 2000 (2000-07), pages 2835-2843, XP002176480 ISSN: 1078-0432

D6: HO MITCHELL ET AL: "In vitro antibody evolution targeting germline hot spots to increase activity of an anti-CD22 immunotoxin." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 7 JAN 2005, vol. 280, no. 1, 7 January 2005 (2005-01-07), pages 607-617, XP002326657 ISSN: 0021-9258

2. Subject-matter of the application

Present application relates to mutated anti-CD22 antibodies and immuno-conjugates. These antibodies have a variable light chain (V_L) wherein CDR1 has a sequence selected from the group consisting of SEQ ID NOs: 7, 8, 9, and 10. The antibody can further comprise a variable heavy chain (V_H) comprising 3 CDRs, wherein the CDR1 has the sequence of SEQ ID NO: 13, the CDR2 has the sequence of SEQ ID NO:15, and the CDR3 has a sequence selected from the group consisting of SEQ ID Nos: 15, 16, 17, 18, and 19. The antibodies of the application are derived from the known RFB4 antibody which has been mutated at positions 30 and 31 of CDR1 of the VL chain of RFB4. These mutants were discovered in the course of in vitro affinity maturation studies. Screening of a phage-display library targeting a hot spot within the light chain CDR1 (L-CDR1) of HA22 identified four mutant phage antibodies that had binding affinity for CD22-positive cells higher than the starting molecule (HA22). The antibodies disclosed in present invention differ from the antibodies disclosed in D1 in only two positions, namely positions 30 and 31 of CDR1 of the V_L chain of RFB4.

3. Novelty

The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-74 appears to be new in the sense of Article 33(2) PCT.

4. Inventive step

4.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-74 does not involve an inventive step in the sense of Article 33(3) PCT.

4.2. The document D1 is regarded as being the closest prior art to the subject-matter of claim 1. D1 relates to mutated anti-CD22 antibodies with increased affinity to CD22-expressing leukemia cells compared to the known anti-CD22 antibody RFB4. In order to increase the affinity of RFB4, phage display and hot spot mutagenesis in VH CDR3 was carried out and RFB4 scFVs with improved binding affinity for CD22 selected (Example 1, Table 1). The deduced amino acid residues of the region mutated in VH CDR3 are shown in Table 2. D1 moreover discloses the RFB4 heavy and light chain amino acid and nucleotide sequences (Fig.1).

The subject-matter of claim 1 differs from D1 in that it relats to anti-CD22 antibodies having a variable light (V_L) chain comprising three complementary determining regions designated CDRs 1, 2, and 3, wherein said CDR1 has a sequence selected from the group consisting of SEQ ID NO: 7, 8, 9, and 10. HA22 antibodies with these CDR1 sequences were shown to bind CD22 with increased affinity (Example 1).

4.3. The problem to be solved by the present invention may therefore be regarded as the provision of further anti-CD22 antibodies that bind CD22 with increased affinity.

4.4. The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons.

D2 discloses (abstract) that in order to increase the affinity of RFB4, phage display and hot spot mutagenesis in V_H CDR3 was carried out. The deduced amino acid residues of the region mutated in V_H CDR3 are shown in Table 2. Mutants GTTW, GYNW, and GTHW had an increase in affinity of 3.5-, 8.5-, and 14-fold, respectively

(page 1000, right hand column, lines 6-9). According to the authors of D2 (page 1001, right hand column, second paragraph) targeting hot spots is an effective method to increase the affinity of the Fv of immunotoxin RFB4 (FV)-PE38. They also disclos that RFB4 is the third antibody in which targeting hot spots has been effective, which indicates that targeting hot spots is a general method that can be used to increase the affinity of antibodies.

D3 disclose that antibody affinity can be improved in vitro by using phage display technology. DNA sequences in the variable regions that are naturally prone to hypermutations were identified, a few hot spots enncoding nonconserved amino acids selected, and random mutations introduced to make libraries with a size requirement between 10^3 and 10^4 independent clones. Panning of the hot spot libraries yielded several mutants with a 15- to 55-fold increase in affinity compared with a single clone with a fourfold increased affinity from a library in which mutagenesis was done outside the hot spots. According to the authors of D3 the strategy should be generally applicable for the rapid isolation of higher-affinity mutants of Fvs, Fabs, and other recombinant antibodies from antibody phage libraries that are small in size.

D4 also discloses (page 9, line 29 - page 11, line 2) a method which permits the generation of Fvs with increased affinity from a small library of variants. This method is based on the fact that the DNA encoding the variable regions of antibodies contain mutational hotspots, which are nucleotide sequences where mutations are frequently concentrated during the in vivo affinity maturation process. Hotspots in the CDRs were targeted and random mutations created. According to D4 any CDR could be altered in this approach. The anti-mesothelin antibody SSscFV was used as a model antibody in D4 beacuse immunotoxins using it as a targeting moiety have shown good effect on human tumors in animal models (page 24, lines 3-6).

D5 likewise discloses (abstract) that targeting hot spots in the CDRs of Fvs is an effective approach to obtaining Fvs with increased affinity. MR1(Fv)-PE38, a single-chain recombinant immunotoxin that targets a mutant form of the epidermal growth factor receptor that is frequently expressed in malignant glioblastomas, was used as a model antibody.

The skilled person faced with the above indicated problem and knowing from D1 that mutation of CDR3 has let to an increase of the affinity of the known anti-CD22 antibody RFB4 would, based on the teaching of any of D2-D5, be motivated to target hotspots in a different CDR of RFB4 in order to obtain anti-CD22 antibodies that bind CD22 with increased affinity. Based on the teaching of any of D2-D5 he would also have a reasonable expectation of success.

The particular CDR1 sequences and thus the antibodies of claim 1 are considered to represent nothing more but an arbitrary selection of all the possible mutants the skilled person would obtain when carrying out an affinity maturation of the known RFB4 antibody according to the method thaught in any of D2-D5.

4.5. None of the remaining claims adds any technical feature that could justify recognition of an inventive step either.

D1 already discloses the particular CDR squences recited in claims 3, 5, 6 and the sequence of the V_H chain of claim 7 (see Table 2 and Fig.1).
D1 moreover discloses chimeric molecules comprising the anti-CD22 antibody and a therapeutic moiety or label, wherein the therapeutic moiety can be a drug or a cytotoxin. Accoding to D1 the cytotoxin can be PE35, PE38, PE38KDEL, PE40, PE4E, and PE38QQR. D1 moreover discloses methods of inhibiting growth of $CD22^+$ cancer cells, methods for detecting the presence of a $CD22^+$ cancer cell in a biological sample, nucleic acids, expression vectors and kits (page 3, line 17 - page 6, line 12).